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(57) Abstract

A novel P-selectin ligand glycoprotein in disclosed, characterized by the amino acid sequence set forth in SEQ ID NO:1 or by the amino acid sequence set forth in SEQ ID NO:3. DNA sequences encoding the P-selectin ligand protein are also disclosed, along with vectors, host cells, and methods of making the P-selectin ligand protein. Pharmaceutical compositions containing the P-selectin ligand protein and methods of treating inflammatory disease states characterized by P-selectin-mediated intercellular adhesion are also disclosed.

TITLE OF THE INVENTION

NOVEL P-SELECTIN LIGAND PROTEIN

BACKGROUND OF THE INVENTION

The present invention relates to the field of anti-inflammatory substances which act by inhibiting leukocyte adhesion to endothelial cells. More particularly, the present invention is directed to a novel ligand for the mammalian adhesion protein known as "P-selectin."

During inflammation leukocytes adhere to the vascular endothelium and enter subendothelial tissue, an interaction which is mediated by specific binding of the selectin or LEC-CAM class of proteins to ligands on target cells. Such selectin-mediated cellular adhesion also occurs in thrombotic disorders and parasitic diseases and may be implicated in metastatic spread of tumor cells.

The selectin proteins are characterized by a N-terminal lectin-like domain, an epidermal growth factor-like domain, and regions of homology to complement binding proteins. Thus far three human selectin proteins have been identified, E-selectin (formerly ELAM-1), L-selectin (formerly LAM-1) and P-selectin (formerly PADGEM or GMP-140). E-selectin is induced on endothelial cells several hours after activation by cytokines, mediating the calcium-dependent interaction between neutrophils and the endothelium. L-selectin is the lymphocyte homing receptor, and P-selectin rapidly appears on the cell surface of platelets when they are activated, mediating calcium-dependent adhesion of neutrophils or monocytes to platelets. P-selectin is also found in the Weibel-Palade bodies of endothelial cells; upon its release from these vesicles P-selectin mediates early

P-selectin binds to carbohydrates containing the non-sialylated form of the Lewis^x blood group antigen and with higher affinity to sialylated Lewis^x. P-selectin may also recognize sulfatides, which are heterogeneous 3-sulfated galactosyl ceramides, isolated from myeloid and tumor cells by lipid extraction. However, the binding of cells bearing P-selectin to cells bearing P-selectin ligands is abolished when the ligand-bearing cells are treated with proteases, indicating that the P-selectin ligand may be a glycoprotein.

Two putative glycoprotein ligands for P-selectin have recently been identified, one of which has been partially purified, (Moore et al., J. Cell Biol. 118, 445-456 (1992)). However, neither amino acid composition nor the amino acid sequence of these glycoproteins are disclosed.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a composition comprising an isolated DNA sequence encoding a P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 402. Also provided is a composition comprising an isolated DNA sequence encoding a soluble P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 310. The invention further provides a composition comprising an isolated DNA sequence encoding a mature P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 402. In another embodiment, the invention provides a composition comprising an isolated DNA sequence encoding a soluble mature P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 310. In another embodiment, the invention provides a composition comprising an isolated DNA sequence encoding a P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:3. The invention further provides a composition comprising

In another embodiment, the invention provides a method of identifying an inhibitor of P-selectin-mediated intercellular adhesion which comprises

(a) combining a P-selectin protein with a P-selectin ligand protein characterized by an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 402, the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 402, the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 310, and the amino acid sequence set forth in SEQ ID NO:3, said combination forming a first binding mixture;

(b) measuring the amount of binding between the P-selectin protein and the P-selectin ligand protein in the first binding mixture;

(c) combining a compound with the P-selectin protein and the P-selectin ligand protein to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

(e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture; wherein the compound is capable of inhibiting P-selectin-mediated intercellular adhesion when a decrease in the amount of binding of the second binding mixture occurs.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have for the first time identified and isolated a novel DNA which encodes a protein which acts as a ligand for P-selectin on human endothelial cells and platelets. The complete amino acid sequence of the P-selectin ligand protein (i.e., the mature peptide plus the leader sequence) is characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 402. Hydrophobicity analysis and comparison with known cleavage patterns predict a signal sequence of 20 to 22 amino acids, i.e., amino acids 1 to 20 or amino acids 1 to 22 of SEQ ID NO:1. The P-selectin ligand protein contains

translationally modified. As expressed in COS and CHO cells, full length P-selectin ligand protein (amino acids 1 to 402 of SEQ ID NO:1 or amino acids 42 to 402 of SEQ ID NO:1) is a homodimeric protein having an apparent molecular of 220 kD as shown by non-reducing SDS-polyacrylamide gel electrophoresis.

Three regions of the P-selectin ligand protein of SEQ ID NO:1 are: an extracellular domain (from about amino acid 21 to 310 of SEQ ID NO:1), a transmembrane domain (from about amino acid 311 to 332 of SEQ ID NO:1), and an intracellular, cytoplasmic domain (from about amino acid 333 to 402 of SEQ ID NO:1). The extracellular domain contains three consensus tripeptide sites (Asn-X-Ser/Thr) of potential N-linked glycosylation beginning at Asn residues 65, 111, and 292. The extracellular domain further contains three potential sites of tyrosine sulfation at residues 46, 48, and 51. The region comprised of residues 55-267 contains a high percentage of proline, serine, and threonine including a subdomain of fifteen decameric repeats of the ten amino acid consensus sequence Ala-Thr/Met-Glu-Ala-Gln-Thr-Thr-X-Pro/Leu-Ala/Thr, wherein X can be either Pro, Ala, Gln, Glu, or Arg. Regions such as these are characteristic of highly O-glycosylated proteins.

COS or CHO cells co-transfected with a gene encoding the P-selectin ligand protein and a gene encoding an (α1,3/1,4) fucosyltransferase (hereinafter 3/4FT) are capable of binding to CHO cells expressing P-selectin on their surface, but are not capable of binding to CHO cells which do not express P-selectin on their surface. In order to bind to P-selectin, either in purified form or expressed on the surface of CHO cells, the gene encoding the P-selectin ligand protein must be co-transfected with the gene encoding a 3/4FT, since transfection of either gene in the absence of the other either abolishes or substantially reduces the P-selectin binding activity. The binding of the P-selectin ligand protein of the invention to P-selectin can be inhibited by EDTA or by a neutralizing monoclonal antibody specific for P-selectin. The binding of the P-selectin ligand protein of the invention to P-selectin is not inhibited by a non-neutralizing monoclonal antibody specific for P-selectin or by

intercellular adhesion in addition to P-selectin-mediated intercellular adhesion.

Fragments of the P-selectin ligand protein which are capable of interacting with P-selectin or which are capable of inhibiting P-selectin-mediated intercellular adhesion are also encompassed by the present invention. Such fragments comprise amino acids 21 to 54 of SEQ ID NO:1, a region of the P-selectin ligand protein having a low frequency of serine and threonine residues; amino acids 55 to 127 of SEQ ID NO:1, having a high frequency of proline, serine, and threonine in addition to two consensus sequences for asparagine-linked glycosylation (Asn-X-Ser/Thr); another larger fragment, amino acids 128 to 267 of SEQ ID NO:1, having both a high frequency of proline, serine, and threonine and containing fifteen repeats of the following ten amino acid consensus sequence: Ala-(Thr/Met)-Glu-Ala-Gln-Thr-Thr-(Pro/Arg/Gln/Ala/Glu)-(Leu/Pro)-(Ala/Thr) (smaller fragments within this large fragment may also retain the capacity to interact with P-selectin or act as inhibitors of P-selectin-mediated intercellular adhesion); the region containing a consensus sequence for asparagine-linked glycosylation and comprising amino acids 268 to 308 of SEQ ID NO:1; the hydrophobic region of the protein represented by amino acids 309 to 333 of SEQ ID NO:1; and the amphiphilic region of the P-selectin ligand protein from amino acids 334 to 402. Additional fragments may comprise amino acid 43 to amino acid 56 of SEQ ID NO:1, with one or more sulfated tyrosines at amino acid 46, amino acid 48, and/or amino acid 51. Fragments of the P-selectin ligand protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. For the purposes of the present invention, all references to "P-selectin ligand protein" herein include fragments capable of binding to P-selectin.

Such fragments may be fused to carrier molecules such as immunoglobulins, to increase the valency of P-selectin ligand binding sites. For example, soluble forms of the P-selectin

form of the P-selectin ligand protein which lacks the signal sequence and which is characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 21 to amino acid 402. In yet another embodiment, the DNA sequence of the invention encodes the mature P-selectin ligand protein characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 402. Another embodiment of the DNA sequence of the invention encodes a soluble form of the P-selectin ligand protein characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 310. The DNA of the invention is also embodied in a DNA sequence encoding a soluble form of the mature P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 310. The DNA of the invention is further embodied in a DNA sequence encoding a soluble form of the P-selectin ligand protein which lacks the signal sequence, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 21 to amino acid 310. The DNA of the present invention is free from association with other human DNAs and is thus characterized as an isolated DNA. As detailed above, DNAs which encode P-selectin ligand fragments which interact with P-selectin are also included in the present invention.

The expression of P-selectin ligand protein mRNA transcripts has been observed in a variety of human cell lines (HL-60, THP-1, U937) and in human monocytes and polymorphonuclear leukocytes by Northern analysis using a P-selectin ligand protein cDNA probe. In all of these cell lines, a major transcript of 2.5 kb was observed. A minor species of approximately 4 kb was observed in the HL60 and U937 cell lines and in polymorphonuclear leukocytes. In contrast, no P-selectin ligand mRNA expression was detected in the human hepatoblastoma cell line HepG2.

The P-selectin ligand protein of the invention is encoded by a single copy gene and is not part of a multi-gene family, as determined by Southern blot analysis. The genomic form of the P-selectin ligand protein of the invention contains a large intron of approximately 9 kb located at nucleotide 54 in the 5'

DNA sequence of SEQ ID NO:1 and which encode proteins having P-selectin ligand protein activity; DNAs which differ from the DNA of SEQ ID NO:1 by virtue of degeneracy of the genetic code; and the variations of the DNA sequence of SEQ ID NO:1 set forth above. Similarly, all references to the "DNA of SEQ ID NO:3" include in addition to the specific DNA sequence set forth in SEQ ID NO:3, DNA sequences encoding the mature P-selectin ligand protein of SEQ ID NO:3; DNA sequences encoding fragments of the P-selectin ligand protein of SEQ ID NO:3 which are capable of binding to P-selectin; DNA sequences encoding soluble forms of the P-selectin ligand protein of SEQ ID NO:3; allelic variations of the DNA sequence of SEQ ID NO:3; DNAs which hybridize to the DNA sequence of SEQ ID NO:3 and which encode proteins having P-selectin ligand protein activity; DNAs which differ from the DNA of SEQ ID NO:3 by virtue of degeneracy of the genetic code; and the variations of the DNA sequence of SEQ ID NO:3 set forth above.

A DNA encoding a soluble form of the P-selectin ligand protein may be prepared by expression of a modified DNA in which the regions encoding the transmembrane and cytoplasmic domains of the P-selectin ligand protein are deleted and/or a stop codon is introduced 3′ to the codon for the amino acid at the carboxy terminus of the extracellular domain. For example, hydrophobicity analysis predicts that the P-selectin ligand protein set forth in SEQ ID NO:1 has a transmembrane domain comprised of amino acids 311 to 332 of SEQ ID NO:1 and a cytoplasmic domain comprised of amino acids 333 to 402 of SEQ ID NO:1. A modified DNA as described above may be made by standard molecular biology techniques, including site-directed mutagenesis methods which are known in the art or by the polymerase chain reaction using appropriate oligonucleotide primers. Methods for producing several DNAs encoding various soluble P-selectin ligand proteins are set forth in Example 5.

The isolated DNA of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the P-selectin ligand

vectors is within the level of ordinary skill in molecular biology. Methods for co-transformation and transformation are also known.

Many DNA sequences encoding PACE are known. For example, a DNA encoding one form of PACE, known as furin, is disclosed in A.M.W. van den Ouweland et al., Nucl. Acids Res. 18, 664 (1990), incorporated herein by reference. A cDNA encoding a soluble form of PACE, known as PACESOL, is set forth in SEQ ID NO:5. DNAs encoding other forms of PACE also exist, and any such PACE-encoding DNA may be used to produce the soluble mature P-selectin ligand protein of the invention, so long as the PACE is capable of cleaving the P-selectin ligand protein at amino acids 38-41. Preferably, a DNA encoding a soluble form of PACE is used to produce the soluble mature P-selectin ligand protein of the present invention.

The DNAs encoding a soluble form of the P-selectin ligand protein and PACE, separately or together, may be operably linked to an expression control sequence such as those contained in the pMT2 or pED expression vectors discussed above, in order to produce the PACE-cleaved soluble P-selectin ligand recombinantly. Additional suitable expression control sequences are known in the art. Examples 3(C) and 3(D) below set forth methods for producing the soluble mature P-selectin ligand protein of the invention.

A number of types of cells may act as suitable host cells for expression of the P-selectin ligand protein. Suitable host cells are capable of attaching carbohydrate side chains characteristic of functional P-selectin ligand protein. Such capability may arise by virtue of the presence of a suitable glycosylating enzyme within the host cell, whether naturally occurring, induced by chemical mutagenesis, or through transfection of the host cell with a suitable expression plasmid containing a DNA sequence encoding the glycosylating enzyme. Host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell

which are characterized by somatic or germ cells containing a DNA sequence encoding the P-selectin ligand protein.

The P-selectin ligand protein of the invention may be prepared by culturing transformed host cells under culture conditions necessary to express a P-selectin binding glycoprotein. The resulting expressed glycoprotein may then be purified from culture medium or cell extracts. Soluble forms of the P-selectin ligand protein of the invention can be purified by affinity chromatography over Lentil lectin-Sepharose® and subsequent elution with 0.5M α-methyl-mannoside. The eluted soluble P-selectin ligand protein can then be further purified and concentrated by a 0-70% ammonium sulfate precipitation step. The protein is then recovered, resuspended, and further purified by size exclusion chromatography over a TSK G4000SW$_{XL}$. Alternatively, full length forms of the P-selectin ligand protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100. The detergent extract can then be passed over an affinity column comprised of immobilized P-selectin, and the P-selectin ligand protein can be eluted from the column with 10mM EDTA in a buffer containing 0.1% detergent. The material eluted from the affinity column can then be dialyzed to remove EDTA and further purified over a Lentil lectin-Sepharose® affinity column, again eluting with 0.5M α-methyl-mannoside.

Alternatively, the P-selectin ligand protein of the invention is concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or

be used to treat hemodialysis and leukophoresis patients. Additionally, the isolated P-selectin ligand protein may be used as an antimetastatic agent. The isolated P-selectin ligand protein may be used itself as an inhibitor of P-selectin-mediated intercellular adhesion or to design inhibitors of P-selectin-mediated intercellular adhesion. The present invention encompasses both pharmaceutical compositions containing isolated P-selectin ligand protein and therapeutic methods of treatment or use which employ the isolated P-selectin ligand protein.

The isolated P-selectin ligand protein, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to the P-selectin ligand protein and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, G-CSF, Meg-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with the isolated P-selectin ligand protein, or to minimize side effects caused by the isolated P-selectin ligand protein. Conversely, the isolated P-selectin ligand protein may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic

simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the isolated P-selectin ligand protein in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of the isolated P-selectin ligand protein used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of isolated P-selectin ligand protein is administered orally, the isolated P-selectin ligand protein will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% isolated P-selectin ligand protein, and preferably from about 25 to 90% isolated P-selectin ligand protein. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the isolated P-selectin ligand protein and preferably from about 1 to 50% isolated P-selectin ligand protein.

When a therapeutically effective amount of isolated P-selectin ligand protein is administered by intravenous, cutaneous or subcutaneous injection, the isolated P-selectin ligand protein will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally

The isolated P-selectin ligand protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the P-selectin ligand protein and which may inhibit P-selectin-mediated cellular adhesion. Such antibodies may be obtained using the entire P-selectin ligand protein as an immunogen, or by using fragments of the P-selectin ligand protein such as the soluble mature P-selectin ligand protein. Smaller fragments of the P-selectin ligand protein may also be used to immunize animals, such as the fragments set forth below: amino acid 42 to amino acid 56 of SEQ ID NO:1 and amino acid 127 to amino acid 138 of SEQ ID NO:1. An additional peptide immunogen comprises amino acid 238 to amino acid 248 of SEQ ID NO:1, with an alanine residue added to the amino terminus of the peptide. Another peptide immunogen comprises amino acid 43 to amino acid 56 of SEQ ID NO:1 having a sulfated tyrosine in any or all of positions 46, 48 or 51. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Monoclonal antibodies binding to the P-selectin ligand glycoprotein or to complex carbohydrate moieties characteristic of the P-selectin ligand glycoprotein may be useful diagnostic agents for the immunodetection of inflammatory diseases and some forms of cancer. Some cancerous cells, such as small cell lung carcinomas, may express detectable levels of the P-selectin ligand protein. This abnormal expression of the P-selectin ligand protein by cancer cells may play a role in the metastasis of these cells.

Neutralizing monoclonal antibodies binding to the P-selectin ligand glycoprotein or to complex carbohydrates characteristic of the P-selectin ligand glycoprotein may also be useful therapeutics for both inflammatory diseases and also in the

Any P-selectin ligand protein may be used in the screening assays described above. For example, the full-length P-selectin ligand protein set forth in SEQ ID NO:1 from amino acid 1 to amino acid 402 may be used to screen for inhibitors; or the mature P-selectin ligand protein set forth in SEQ ID NO:1 from amino acid 42 to amino acid 402 may be used to screen for inhibitors, or the soluble mature P-selectin ligand protein set forth in SEQ ID NO:1 from amino acid 42 to amino acid 310 may be used to screen for inhibitors. Alternatively, the P-selectin ligand protein of SEQ ID NO:3 from amino acid 1 to amino acid 412, or a mature form of the P-selectin ligand protein as set forth in SEQ ID NO:3 from amino acid 42 to amino acid 412, or a soluble mature form of the P-selectin ligand protein set forth in SEQ ID NO:3 from amino acid 42 to amino acid 320 may be used to screen for inhibitors of intercellular adhesion in accordance with the present invention.

In such a screening assay, a first binding mixture is formed by combining P-selectin and the P-selectin ligand protein, and the amount of binding in the first binding mixture (B_o) is measured. A second binding mixture is also formed by combining P-selectin, the P-selectin ligand protein, and the compound or agent to be screened, and the amount of binding in the second binding mixture (B) is measured. The amounts of binding in the first and second binding mixtures are compared, for example, by performing a B/B_o calculation. A compound or agent is considered to be capable of inhibiting P-selectin mediated intercellular adhesion if a decrease in binding in the second binding mixture as compared to the first binding mixture is observed. The formulation and optimization of binding mixtures is within the level of skill in the art, such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding, and additional control assays may be included in the screening assay of the invention.

Compounds found to reduce by at least about 10%, preferably greater than about 50% or more of the binding activity of P-selectin ligand protein to P-selectin may thus be identified and then secondarily screened in other selectin binding assays,

EXAMPLE 1

CLONING OF THE P-SELECTIN LIGAND PROTEIN GENE

A. Construction of the HL60 cDNA library

An HL60 cDNA library was constructed for expression cloning the P-selectin ligand. PolyA$^+$ RNA was isolated from total RNA from the human promyelocytic cell line HL60 (S.J. Collins, et al., *supra*) using a Fast Track mRNA Isolation Kit (Invitrogen; San Diego, CA). Double stranded cDNA was synthesized from the polyA$^+$ RNA fraction and blunt-end ligated with EcoRI adaptors (5'-AATTCCGTCGACTCTAGAG-3',5'CTCTAGAGTCGACGG-3'). The cDNA was ligated into the expression vector pMT21 (R. Kaufman et al., J. Mol. Cell. Biol. 9, 946-958 (1989) that had been incubated sequentially with EcoRI endonuclease and calf intestinal alkaline phosphatase and gel purified. The ligation product was electroporated in 2 μl aliquots into competent E. coli DH5α cells and grown in 1 ml of SOB medium (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, New York, Cold Spring Harbor Laboratory Press, p1.90 (1989)) which has been supplemented with 10 mM $MgCl_2$, 10 mM $MgSO_4$, and 2 % glycerol for one hour at 37°C. In order to divide the library into smaller subsets, an aliquot from each ml of bacterial suspension was plated onto agar plates in the presence of ampicillin, and the number of colonies per ml was calculated. Assuming that each colony represented one cDNA clone, 600,000 clones were generated and divided into subsets of approximately 16,000 clones per pool. Each of the 38 pools were grown overnight in L-broth in the presence of ampicillin and the plasmids were purified over a CsCl gradient.

B. Screening for the P-selectin ligand protein gene

In the first stage,the LEC-γ1 binding assay of Example 4(A) was utilized to pan the HL60 cDNA library and thereby to enrich for the plasmid of interest. Six μg of each HL60 cDNA library pool was co-transfected with 2 μg of α 3/4FT gene (Example 2) into COS cells. Approximately 45 hours post-transfection, the COS cells were lifted from the plates by incubating the cells in

EXAMPLE 2

CLONING THE α 1,3/1,4 FUCOSYLTRANSFERASE GENE

The α 1,3/1,4 fucosyltransferase gene (3/4FT) was cloned from total human genomic DNA (Clontech Laboratories) by means of PCR. The sense oligonucleotide primer contained an XbaI site and the 5'terminus of the gene (5'-TAGCATACGCTCTAGAGCATGGATCCCCTGGGTGCA
GCCAAGC-3'), and the antisense oligonucleotide primer contained an EcoRI site and the 3'terminus of the gene (5'-CCGGAATTCTCAGGTGAA
CCAAGCCGC-3'). The PCR product was sequentially digested with XbaI and EcoRI and purified by standard gel purification methods. This gene was then ligated with vector pMT3Sv2ADA (R. Kaufman, Methods in Enzymology, *supra*) that had also been sequentially digested with XbaI and EcoRI and purified by standard gel purification methods. Competent HB101 cells (Biorad) were transformed with this ligation product and then plated on agar plates in the presence of ampicillin. Nitrocellulose filter lifts of ampicillin-resistant transformants were probed with a radiolabeled oligonucleotide (5'-AAGTATCTGTCCAGGGCTTCCAGGT-3') complementary to the nucleotide region 506-530 in the middle of the gene (J. Sambrook et al., *supra*).

Plasmid DNA minipreps were prepared from twelve positive clones. The purified DNA was then digested with EcoRI and XbaI to identify the correct clone with the proper size insert. This clone (pEA.3/4FT) was then grown up large scale and the DNA isolated by CsCl density gradient banding (J. Sambrook et al., *supra*). DNA sequencing confirmed the identity of the 3/4FT gene. The functionality of the gene was assessed in a cell-cell binding assay as follows. COS-1 monkey cells [(clone M6; M. Horwitz et al., Mol. Appl. Genet., 2:147-149, (1983)] were transfected with 3/4FT using DEAE dextran followed by DMSO shock treatment and chloroquine incubation [L. Sompeyrac and K. Dana, Proc. Natl. Acad. Sci., 78:7575-7578 (1981); M. Lopata et al., Nucleic Acids Res., 12:5707-5717, (1984); H. Luthman and G. Magnuson, Nucleic Acids Res., 11:1295-1308, (1983)]. The transfected COS cells

with pED.sPSL.T7 and pEA.3/4 FT. At forty-five hr post-transfection, the COS cells were labeled with 250 μCi/ml ^{35}S methionine (NEN) for 5 hours and the medium was collected. Expression of sPSL.T7 protein was confirmed by immunoprecipitation with anti-T7 antibodies.

C. **Expression of PACE-cleaved P-selectin ligand in COS Cells**

COS cells were co-transfected with the pED.sPSL.T7 plasmid of Example 5(C), the pEA.3/4FT cDNA of Example 2, and a plasmid containing the PACE cDNA as set forth in SEQ ID NO:5. A parallel control co-transfection was done using only the pED.sPSL.T7 plasmid and the pEA.3/4FT plasmid. After 45 hours, conditioned medium from these transfected COS cells was coated onto plastic dishes and binding to CHO:P-selectin cells (Example 4) was determined. An approximately two-fold increase in bound CHO:P-selectin cells was observed for dishes coated with medium containing the P-selectin ligand co-expressed with PACE, as compared with medium containing P-selectin ligand which had not been co-expressed with PACE. Amino acid sequencing of the N-terminus of purified sPSL.T7 protein from the PACE co-transfection showed that all of the ligand had been cleaved at the PACE consensus site (amino acids 38-41 of SEQ ID NO:1). Radiolabeling of co-transfected COS cells with ^{35}S-methionine and subsequent SDS-polyacrylamide gel electrophoresis and autoradiography showed that comparable quantities of the P-selectin ligand had been secreted in both co-transfections.

D. **Expression of the P-selectin Ligand Protein in CHO Cells**

A full-length form (amino acids 1-402) of the P-selectin ligand protein was expressed in the CHO(DUKX) cell line (Urlaub & Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220 (1980)) as follows: approximately 25 μg of the pMT21:PL85 plasmid and approximately 8 μg of the pED.3/4FT (produced by restriction of pEA.3/4FT with EcoRI and XbaI and insertion of the resulting fragment into the pED plasmid) were co-transfected into CHO(DUKX) cells using the calcium phosphate method. Transfectants were selected for resistance to methotrexate. After two weeks,

specific monoclonal antibody or the LEC-γ1 chimera of Example 4(A). In a similar fashion, a stable cell line expressing the mature full length form (amino acids 42-402) of the P-selectin ligand protein was obtained by co-transfection of pMT21:PL85and pED.3/4FT into the CHO-PACE line.

Stable cell lines expressing the sPSL.Q protein of Example 5(B) and the sPSL.Fc protein of Example 5(D) were constructed as follows: plasmids pED.sPSL.Q (25 μg) or pED.sPSL.Fc (25 μg) were cotransfected with approximately 25 μg of the pED.3/4FT plasmid described above and approximately 20 μg of a plasmid containing the PACE cDNA as set forth in SEQ ID NO:5) as well as the neomycin resistance gene into CHO(DUKX) cells using the calcium phosphate method. Transfectants were selected for resistance to methotrexate and the G418 antibiotic. Approximately two weeks later, individual colonies were screened for SLe^x expression using sRBC/CSLEX-1 conjugate binding. The positive colonies were picked in G418 medium at 1 mg/ml concentration. After 2-3 weeks in culture, cells were amplified with methotrexate in a stepwise selection. The stable cell lines obtained were designated CD-sPSL.Q (R8.2) and CD-sPSL.Fc (R8.1), respectively. The expression of sPSL.Q and sPSL.Fc protein was confirmed by standard immunoprecipitation method using the anti P-selectin ligand protein polyclonal antibody of Example 7(A).

EXAMPLE 4

ASSAYS OF P-SELECTIN-MEDIATED INTERCELLULAR ADHESION

A. LEC-γ1 Binding Assay

A DNA encoding a chimeric form of P-selectin conjugated to the Fc portion of a human IgGγ1 (LEC-γ1) was constructed using known methods (Aruffo et al. Cell 67, 35-44 (1991)), and stably transfected into dhfr$^-$ CHO cells (CHO DUKX) for high level production of the chimeric LEC-γ1 protein, which was purified for use in the binding assay set forth below.

Petri dishes were coated first with a polyclonal anti-human IgGγ1 Fc antibody and then with LEC-γ1. This method orients the

cells/ml for 30 minutes at 4°C in α medium containing 1% BSA (control); α medium containing 1% BSA, 5 mM EDTA and 5 mM EGTA; α medium containing 1% BSA and 10 μg/ml of a neutralizing anti P-selectin monoclonal antibody; and α medium containing 1% BSA and a non-neutralizing anti-P-selectin monoclonal antibody. The preincubated cells were then added to the wells containing the transfected COS cells. After a 10 minute incubation, unbound cells were removed by 4 changes of medium. The bound CHO:P-selectin cells were released by trypsinization and quantified by scintillation counting.

COS cells co-transfected with P-selectin ligand and the 3/4FT induced approximately 5.4-fold more binding of CHO:P-selectin cells relative to COS mock cells; assay in the presence of EGTA and EDTA reduced binding to the level of the mock transfected COS cells. Likewise, incubation with neutralizing anti-P-selectin antibody also eliminated specific binding, whereas non-neutralizing antibody had no effect. In contrast, the binding of CHO:P-selectin to COS cells transfected with P-selectin ligand alone was not statistically different than binding to the mock-transfected COS in both the presence or absence of EDTA and EGTA, or anti-P-selectin antibodies. The binding of CHO:P-selectin cells to COS cells transfected with 3/4 FT alone was approximately 2-fold greater than to the mock-transfected COS, but was unaffected by the presence or absence of EDTA and EGTA.

EXAMPLE 5

CONSTRUCTION OF SOLUBLE P-SELECTIN LIGANDS

The EcoRI adaptors used to generate the cDNA library from HL60 cells in Example I contain an XbaI restriction site (TCTAGA) just 5′ of the beginning of SEQ ID NO:1 as it is located in the pMT21:PL85 plasmid. In order to generate soluble forms of the PSL, the pMT21:PL85 plasmid was restricted with XbaI and with HincII (which cleaves after nucleotide 944 of SEQ ID NO:1). The approximately 950 bp fragment thus generated, containing all of the encoded extracellular segment of the ligand up to and including the codon for valine 295, was isolated and used to

were duplexed and ligated with the large XbaI-EcoRI fragment of mammalian expression plasmid pED. The resulting plasmid, pED.T7 was restricted with XbaI and SmaI and ligated to the 950 bp XbaI-HincII fragment described above, resulting in plasmid pED.sPSL.T7.

The protein resulting from expression of pED.sPSL.T7 was designated sPSL.T7.

D. Construction of Soluble P-selectin Ligand--IgGFc Chimera

The plasmid DNA encoding a soluble, extracellular form of the P-selectin ligand protein fused to the Fc portion of human immunoglobulin IgG1 was constructed as follows: the mammalian expression vector pED.Fc contains sequences encoding the Fc region of a human IgG1 with a novel linker sequence enabling the fusion of coding sequences amino terminal to the hinge region via a unique XbaI restriction site. A three fragment ligation was performed: pED.Fc was restricted with XbaI and gel purified in linear form. The 950 bp fragment from pMT21:PL85 described above comprised the second fragment. The third fragment consisted of annealed synthetic oligonucleotide DNAs having the following sequence:

5′ - CTGCGGCCGCAGT

5′ - CTAGACTGCGGCCGCAG

The ligation products were grown as plasmid DNAs and individual clones having the correct configuration were identified by DNA sequencing. The plasmid was designated pED.PSL.Fc. The DNA coding region of the resulting soluble P-selectin ligand /Fc fusion protein is shown in SEQ ID NO:6.

EXAMPLE 6

CHARACTERIZATION OF EXPRESSED P-SELECTIN LIGANDS

A. Binding Characterization of Full-Length P-selectin Ligand Protein Expressed on COS Cells

Co-transfection of COS cells with the pEA.3/4FT plasmid of Example 2 and the pMT21:PL85 plasmid of Example 1 yields COS cells which specifically bind to CHO:P-selectin cells. This binding is observed only upon co-transfection of pEA.3/4FT and pMT21:PL85; use of either plasmid alone generates COS cells which

1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10% non-fat milk overnight at 4°C. Blots were rinsed once in the above buffer, minus the milk, and incubated in blotting buffer (10 mMMOPS pH 7.5, 0.1M NaCl, 1% bovine serum albumin, 0.05% Thesit, 1 mM $MgCl_2$, 1 mM $CaCl_2$) for 30 minutes at room temperature.

The blots were then probed for the P-selectin ligand as follows: 50 ng of a P-selectin/Fc chimera was pre-incubated with 3 μCi of ^{125}I-Protein A in blotting buffer for 30 minutes at room temperature. Additional excipients (e.g., EDTA, EGTA, monoclonal antibodies) could be added to the pre-incubation mixture at this point to evaluate their effects on binding of the chimera to the P-selectin ligand. The pre-incubated mixture was then incubated with the blots (prepared as above) for 60 minutes at room temperature, and the blots were subsequently washed four times with the same blotting buffer (without bovine serum albumin), air dried, and autoradiographed at -70°C.

Under non-reducing conditions, two bands were observed with this technique for membrane extracts prepared from co-transfected COS cells. The major band migrated with an estimated molecular weight of approximately 220 kD, whereas the minor band migrated with a molecular weight of approximately 110 kD. Under reducing conditions, only a single band was observed with a molecular weight of approximately 110 kD, indicating that under non-reducing conditions, the P-selectin ligand exists as a homodimer. The approximate molecular weight of the reduced monomer is greater than that predicted from the deduced amino acid sequence of the cDNA clone (45 kD), indicating that the expressed protein undergoes extensive post-translational modification (see Example 6(C)). The specificity of the P-selectin/Fc chimera was confirmed by the observation that a nonspecific IgG_1 probe yielded no bands on the blots. Additionally, the binding of the P-selectin/Fc chimera to the blots was abolished by EDTA, EGTA, and a neutralizing anti-P-selectin monoclonal antibody. Specific bands on the blots were observed only from membrane extracts of COS cells co-transfected with the pEA.3/4FT and pMT21:PL85 plasmids. Membrane extracts from control transfections

into two equal fractions. One fraction was precipitated with the P-selectin polyclonal antibody of Example 7(A), to show the effect of digestion on the electrophoretic mobility. The other fraction was precipitated with the LEC-γ1 chimera of Example 4(A), to assess the remaining P-selectin ligand binding activity after digestion. The immunoprecipitationed samples were analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions and autoradiography.

In the absence of glycosidase treatment, autoradiography revealed comparable bands (with molecular weights of 110 kD) for each precipitation. When the P-selectin ligand protein was treated with neuraminidase, anti-P-selectin ligand polyclonal antibody precipitation revealed a slight decrease in mobility, consistent with removal of sialic acid residues. The amount of P-selectin ligand protein precipitated by LEC-γ1 was significantly reduced after neuraminidase treatment, consistent with the role of sialic acid residues in the P-selectin/P-selectin ligand interaction. When the P-selectin ligand protein was treated with both neuraminidase and O-glycanase, a substantial increase in electrophoretic mobility was observed after precipitation with the anti-P-selectin ligand polyclonal antibody, indicating that a number of O-linked oligosaccharide chains had been removed. However, removal of O-linked oligosaccharides from the P-selectin ligand protein may not have been complete, since the electrophoretic mobility did not correspond to a protein with a molecular weight of 38 kD, as would be predicted from the amino acid sequence set forth in SEQ ID NO:1. The neuraminidase/O-glycanase digested P-selectin ligand protein bound to LEC-γ1 very poorly, further indicating the role of oligosaccharides in the P-selectin/P-selectin ligand interaction. Treatment of the purified P-selectin ligand with N-glycanase resulted in a slight increase in electrophoretic mobility, demonstrating that some of the consensus sites for N-linked glycosylation are occupied. The amount of P-selectin ligand protein precipitated by LEC-γ1 was slightly reduced, indicating that N-linked glycosylation also contributes to the

stirring for 30 minutes, the precipitate was resuspended in a minimal volume of TBS (20 mM Tris-HCl, 150 mM NaCl, pH 7.5) and applied to a TSK G4000SW$_{XL}$ gel filtration column equilibrated in TBS. The flow rate on the column was 0.5 ml/min and a guard column was employed. In aliquots of < 250 µl, the resuspended ammonium sulfate pellet was injected on the column and fractions analyzed by SDS-PAGE with Western analysis. Fractions containing sPLS.T7 were pooled and then used for immunizing rabbits.

Antibodies to sPSL.T7 were generated in the standard fashion by antigen priming and subsequent boosting over a 3 month period. Specifically, primary immunization was performed by mixing 50 µg of sPSL.T7 (denatured by mixing in 0.1% SDS and heating for 10 minutes at 100°C) with complete Freund's adjuvant and injected at five sites subcutaneously. The second (and all subsequent) boosts were performed by mixing 25 µg of sPSL.T7 (denatured by mixing in 0.1% SDS and heating for 10 minutes at 100°C) [12.5 µg for the third and subsequent boosts] with incomplete Freund's adjuvant and injecting at two sites subcutaneously (or later, intramuscularly) every two weeks. Test bleeds were performed every two weeks to monitor antibody titer. When the antibody titer reached a suitable level, a larger scale bleed was performed and a total serum fraction prepared. This polyclonal antibody preparation was used to inhibit the specific binding of HL60 cells to CHO:P-selectin cells in a manner similar to that described in Example 4.

This assay employed fluorescently-labeled HL60 cells (labelled with BCECFAM; 2',7'-bis-(2-carboxymethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester) binding to CHO cells plated on the bottom of microtiter plates. The labelled HL60 cells were pre-incubated with either sera containing polyclonal antibody or with pre-immune sera for 30 minutes at 4°C. The cells were then washed and incubated with the CHO:P-selectin cells for 10 minutes. The plates were then washed and the fluorescence read with a fluorescence microtiter plate reader. Using this assay, a 1:15 dilution of the anti-sPSL.T7 polyclonal serum resulted in essentially complete inhibition of HL60 cell binding to CHO:P-selectin. Demonstrable inhibition of HL60

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENETICS INSTITUTE, INC.

(ii) TITLE OF INVENTION: NOVEL P-SELECTIN LIGAND PROTEIN

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Legal Affairs
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02140

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/965,662
(B) FILING DATE: 23-OCT-1992
(C) APPLICATION NUMBER: US 08/112,608
(D) FILING DATE: 26-AUG-1993

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(B) REGISTRATION NUMBER: 33,194
(C) REFERENCE/DOCKET NUMBER: GI 5213B-PCT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1649 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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CGA CTG ACG GCC ACG GAG GCA CAG ACC ACT CCA CTG GCA GCC ACA GAG                539
Arg Leu Thr Ala Thr Glu Ala Gln Thr Thr Pro Leu Ala Ala Thr Glu
145                 150                 155                 160

GCA CAG ACC ACT CCA CCA GCA GCC ACG GAA GCA CAG ACC ACT CAA CCC                587
Ala Gln Thr Thr Pro Pro Ala Ala Thr Glu Ala Gln Thr Thr Gln Pro
                165                 170                 175

ACA GGC CTG GAG GCA CAG ACC ACT GCA CCA GCA GCC ATG GAG GCA CAG                635
Thr Gly Leu Glu Ala Gln Thr Thr Ala Pro Ala Ala Met Glu Ala Gln
            180                 185                 190

ACC ACT GCA CCA GCA GCC ATG GAA GCA CAG ACC ACT CCA CCA GCA GCC                683
Thr Thr Ala Pro Ala Ala Met Glu Ala Gln Thr Thr Pro Pro Ala Ala
        195                 200                 205

ATG GAG GCA CAG ACC ACT CAA ACC ACA GCC ATG GAG GCA CAG ACC ACT                731
Met Glu Ala Gln Thr Thr Gln Thr Thr Ala Met Glu Ala Gln Thr Thr
    210                 215                 220

GCA CCA GAA GCC ACG GAG GCA CAG ACC ACT CAA CCC ACA GCC ACG GAG                779
Ala Pro Glu Ala Thr Glu Ala Gln Thr Thr Gln Pro Thr Ala Thr Glu
225                 230                 235                 240

GCA CAG ACC ACT CCA CTG GCA GCC ATG GAG GCC CTG TCC ACA GAA CCC                827
Ala Gln Thr Thr Pro Leu Ala Ala Met Glu Ala Leu Ser Thr Glu Pro
                245                 250                 255

AGT GCC ACA GAG GCC CTG TCC ATG GAA CCT ACT ACC AAA AGA GGT CTG                875
Ser Ala Thr Glu Ala Leu Ser Met Glu Pro Thr Thr Lys Arg Gly Leu
            260                 265                 270

TTC ATA CCC TTT TCT GTG TCC TCT GTT ACT CAC AAG GGC ATT CCC ATG                923
Phe Ile Pro Phe Ser Val Ser Ser Val Thr His Lys Gly Ile Pro Met
        275                 280                 285

GCA GCC AGC AAT TTG TCC GTC AAC TAC CCA GTG GGG GCC CCA GAC CAC                971
Ala Ala Ser Asn Leu Ser Val Asn Tyr Pro Val Gly Ala Pro Asp His
    290                 295                 300

ATC TCT GTG AAG CAG TGC CTG CTG GCC ATC CTA ATC TTG GCG CTG GTG               1019
Ile Ser Val Lys Gln Cys Leu Leu Ala Ile Leu Ile Leu Ala Leu Val
305                 310                 315                 320

GCC ACT ATC TTC TTC GTG TGC ACT GTG GTG CTG GCG GTC CGC CTC TCC               1067
Ala Thr Ile Phe Phe Val Cys Thr Val Val Leu Ala Val Arg Leu Ser
                325                 330                 335

CGC AAG GGC CAC ATG TAC CCC GTG CGT AAT TAC TCC CCC ACC GAG ATG               1115
Arg Lys Gly His Met Tyr Pro Val Arg Asn Tyr Ser Pro Thr Glu Met
            340                 345                 350

GTC TGC ATC TCA TCC CTG TTG CCT GAT GGG GGT GAG GGG CCC TCT GCC               1163
Val Cys Ile Ser Ser Leu Leu Pro Asp Gly Gly Glu Gly Pro Ser Ala
        355                 360                 365

ACA GCC AAT GGG GGC CTG TCC AAG GCC AAG AGC CCG GGC CTG ACG CCA               1211
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    370                 375                 380

GAG CCC AGG GAG GAC CGT GAG GGG GAT GAC CTC ACC CTG CAC AGC TTC               1259
Glu Pro Arg Glu Asp Arg Glu Gly Asp Asp Leu Thr Leu His Ser Phe
385                 390                 395                 400

CTC CCT TAGCTCACTC TGCCATCTGT TTTGGCAAGA CCCCACCTCC ACGGGCTCTC               1315
Leu Pro
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Thr Glu Ala Gln Thr Thr Pro Leu Ala Ala Thr Glu Ala Gln Thr Thr
130 135 140

Arg Leu Thr Ala Thr Glu Ala Gln Thr Thr Pro Leu Ala Ala Thr Glu
145 150 155 160

Ala Gln Thr Thr Pro Pro Ala Ala Thr Glu Ala Gln Thr Thr Gln Pro
165 170 175

Thr Gly Leu Glu Ala Gln Thr Thr Ala Pro Ala Ala Met Glu Ala Gln
180 185 190

Thr Thr Ala Pro Ala Ala Met Glu Ala Gln Thr Thr Pro Pro Ala Ala
195 200 205

Met Glu Ala Gln Thr Thr Gln Thr Thr Ala Met Glu Ala Gln Thr Thr
210 215 220

Ala Pro Glu Ala Thr Glu Ala Gln Thr Thr Gln Pro Thr Ala Thr Glu
225 230 235 240

Ala Gln Thr Thr Pro Leu Ala Ala Met Glu Ala Leu Ser Thr Glu Pro
245 250 255

Ser Ala Thr Glu Ala Leu Ser Met Glu Pro Thr Thr Lys Arg Gly Leu
260 265 270

Phe Ile Pro Phe Ser Val Ser Ser Val Thr His Lys Gly Ile Pro Met
275 280 285

Ala Ala Ser Asn Leu Ser Val Asn Tyr Pro Val Gly Ala Pro Asp His
290 295 300

Ile Ser Val Lys Gln Cys Leu Leu Ala Ile Leu Ile Leu Ala Leu Val
305 310 315 320

Ala Thr Ile Phe Phe Val Cys Thr Val Val Leu Ala Val Arg Leu Ser
325 330 335

Arg Lys Gly His Met Tyr Pro Val Arg Asn Tyr Ser Pro Thr Glu Met
340 345 350

Val Cys Ile Ser Ser Leu Leu Pro Asp Gly Gly Glu Gly Pro Ser Ala

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GGG GCA GTC ACA GAG CTG ACC ACG GAG CTG GCC AAC ATG GGG AAC CTG          336
Gly Ala Val Thr Glu Leu Thr Thr Glu Leu Ala Asn Met Gly Asn Leu
           100                 105                 110

TCC ACG GAT TCA GCA GCT ATG GAG ATA CAG ACC ACT CAA CCA GCA GCC          384
Ser Thr Asp Ser Ala Ala Met Glu Ile Gln Thr Thr Gln Pro Ala Ala
       115                 120                 125

ACG GAG GCA CAG ACC ACT CAA CCA GTG CCC ACG GAG GCA CAG ACC ACT          432
Thr Glu Ala Gln Thr Thr Gln Pro Val Pro Thr Glu Ala Gln Thr Thr
   130                 135                 140

CCA CTG GCA GCC ACA GAG GCA CAG ACA ACT CGA CTG ACG GCC ACG GAG          480
Pro Leu Ala Ala Thr Glu Ala Gln Thr Thr Arg Leu Thr Ala Thr Glu
145                 150                 155                 160

GCA CAG ACC ACT CCA CTG GCA GCC ACA GAG GCA CAG ACC ACT CCA CCA          528
Ala Gln Thr Thr Pro Leu Ala Ala Thr Glu Ala Gln Thr Thr Pro Pro
               165                 170                 175

GCA GCC ACG GAA GCA CAG ACC ACT CAA CCC ACA GGC CTG GAG GCA CAG          576
Ala Ala Thr Glu Ala Gln Thr Thr Gln Pro Thr Gly Leu Glu Ala Gln
           180                 185                 190

ACC ACT GCA CCA GCA GCC ATG GAG GCA CAG ACC ACT GCA CCA GCA GCC          624
Thr Thr Ala Pro Ala Ala Met Glu Ala Gln Thr Thr Ala Pro Ala Ala
       195                 200                 205

ATG GAA GCA CAG ACC ACT CCA CCA GCA GCC ATG GAG GCA CAG ACC ACT          672
Met Glu Ala Gln Thr Thr Pro Pro Ala Ala Met Glu Ala Gln Thr Thr
   210                 215                 220

CAA ACC ACA GCC ATG GAG GCA CAG ACC ACT GCA CCA GAA GCC ACG GAG          720
Gln Thr Thr Ala Met Glu Ala Gln Thr Thr Ala Pro Glu Ala Thr Glu
225                 230                 235                 240

GCA CAG ACC ACT CAA CCC ACA GCC ACG GAG GCA CAG ACC ACT CCA CTG          768
Ala Gln Thr Thr Gln Pro Thr Ala Thr Glu Ala Gln Thr Thr Pro Leu
               245                 250                 255

GCA GCC ATG GAG GCC CTG TCC ACA GAA CCC AGT GCC ACA GAG GCC CTG          816
Ala Ala Met Glu Ala Leu Ser Thr Glu Pro Ser Ala Thr Glu Ala Leu
           260                 265                 270

TCC ATG GAA CCT ACT ACC AAA AGA GGT CTG TTC ATA CCC TTT TCT GTG          864
Ser Met Glu Pro Thr Thr Lys Arg Gly Leu Phe Ile Pro Phe Ser Val
       275                 280                 285

TCC TCT GTT ACT CAC AAG GGC ATT CCC ATG GCA GCC AGC AAT TTG TCC          912
Ser Ser Val Thr His Lys Gly Ile Pro Met Ala Ala Ser Asn Leu Ser
   290                 295                 300

GTC AAC TAC CCA GTG GGG GCC CCA GAC CAC ATC TCT GTG AAG CAG TGC          960
Val Asn Tyr Pro Val Gly Ala Pro Asp His Ile Ser Val Lys Gln Cys
305                 310                 315                 320

CTG CTG GCC ATC CTA ATC TTG GCG CTG GTG GCC ACT ATC TTC TTC GTG         1008
Leu Leu Ala Ile Leu Ile Leu Ala Leu Val Ala Thr Ile Phe Phe Val
               325                 330                 335

TGC ACT GTG GTG CTG GCG GTC CGC CTC TCC CGC AAG GGC CAC ATG TAC         1056
Cys Thr Val Val Leu Ala Val Arg Leu Ser Arg Lys Gly His Met Tyr
           340                 345                 350

CCC GTG CGT AAT TAC TCC CCC ACC GAG ATG GTC TGC ATC TCA TCC CTG         1104
Pro Val Arg Asn Tyr Ser Pro Thr Glu Met Val Cys Ile Ser Ser Leu
       355                 360                 365
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Thr Glu Ala Gln Thr Thr Gln Pro Val Pro Thr Glu Ala Gln Thr Thr
130 135 140

Pro Leu Ala Ala Thr Glu Ala Gln Thr Thr Arg Leu Thr Ala Thr Glu
145 150 155 160

Ala Gln Thr Thr Pro Leu Ala Ala Thr Glu Ala Gln Thr Thr Pro Pro
165 170 175

Ala Ala Thr Glu Ala Gln Thr Thr Gln Pro Thr Gly Leu Glu Ala Gln
180 185 190

Thr Thr Ala Pro Ala Ala Met Glu Ala Gln Thr Thr Ala Pro Ala Ala
195 200 205

Met Glu Ala Gln Thr Thr Pro Pro Ala Ala Met Glu Ala Gln Thr Thr
210 215 220

Gln Thr Thr Ala Met Glu Ala Gln Thr Thr Ala Pro Glu Ala Thr Glu
225 230 235 240

Ala Gln Thr Thr Gln Pro Thr Ala Thr Glu Ala Gln Thr Thr Pro Leu
245 250 255

Ala Ala Met Glu Ala Leu Ser Thr Glu Pro Ser Ala Thr Glu Ala Leu
260 265 270

Ser Met Glu Pro Thr Thr Lys Arg Gly Leu Phe Ile Pro Phe Ser Val
275 280 285

Ser Ser Val Thr His Lys Gly Ile Pro Met Ala Ala Ser Asn Leu Ser
290 295 300

Val Asn Tyr Pro Val Gly Ala Pro Asp His Ile Ser Val Lys Gln Cys
305 310 315 320

Leu Leu Ala Ile Leu Ile Leu Ala Leu Val Ala Thr Ile Phe Phe Val
325 330 335

Cys Thr Val Val Leu Ala Val Arg Leu Ser Arg Lys Gly His Met Tyr
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Pro Val Arg Asn Tyr Ser Pro Thr Glu Met Val Cys Ile Ser Ser Leu
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ACCCTGGAGG CCAATAAGAA CCTCACATGG CGGGACATGC AACACCTGGT GGTACAGACC 1200

TCGAAGCCAG CCCACCTCAA TGCCAACGAC TGGGCCACCA ATGGTGTGGG CCGGAAAGTG 1260

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TGGACCACAG TGGCCCCCCA GCGGAAGTGC ATCATCGACA TCCTCACCGA GCCCAAAGAC 1380

ATCGGGAAAC GGCTCGAGGT GCGGAAGACC GTGACCGCGT GCCTGGGCGA GCCCAACCAC 1440

ATCACTCGGC TGGAGCACGC TCAGGCGCGG CTCACCCTGT CCTATAATCG CCGTGGCGAC 1500

CTGGCCATCC ACCTGGTCAG CCCCATGGGC ACCCGCTCCA CCCTGCTGGC AGCCAGGCCA 1560

CATGACTACT CCGCAGATGG GTTTAATGAC TGGGCCTTCA TGACAACTCA TTCCTGGGAT 1620

GAGGATCCCT CTGGCGAGTG GGTCCTAGAG ATTGAAAACA CCAGCGAAGC CAACAACTAT 1680

GGGACGCTGA CCAAGTTCAC CCTCGTACTC TATGGCACCG CCCCTGAGGG GCTGCCCGTA 1740

CCTCCAGAAA GCAGTGGCTG CAAGACCCTC ACGTCCAGTC AGGCCTGTGT GGTGTGCGAG 1800

GAAGGCTTCT CCCTGCACCA GAAGAGCTGT GTCCAGCACT GCCCTCCAGG CTTCGCCCCC 1860

CAAGTCCTCG ATACGCACTA TAGCACCGAG AATGACGTGG AGACCATCCG GGCCAGCGTC 1920

TGCGCCCCCT GCCACGCCTC ATGTGCCACA TGCCAGGGGC CGGCCCTGAC AGACTGCCTC 1980

AGCTGCCCCA GCCACGCCTC CTTGGACCCT GTGGAGCAGA CTTGCTCCCG GCAAAGCCAG 2040

AGCAGCCGAG AGTCCCCGCC ACAGCAGCAG CCACCTCGGC TGCCCCCGGA GGTGGAGGCG 2100

GGGCAACGGC TGCGGGCAGG GCTGCTGCCC TCACACCTGC CTGAGTGATG A 2151

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1591 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: sPSL.Fc

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGCCTCTGC AACTCCTCCT GTTGCTGATC CTACTGGGCC CTGGCAACAG CTTGCAGCTG 60

TGGGACACCT GGGCAGATGA AGCCGAGAAA GCCTTGGGTC CCCTGCTTGC CCGGGACCGG 120

AGACAGGCCA CCGAATATGA GTACCTAGAT TATGATTTCC TGCCAGAAAC GGAGCCTCCA 180

CLAIMS

1. A composition comprising an isolated DNA sequence encoding a P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 402.

2. A composition comprising an isolated DNA sequence encoding a soluble P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 310.

3. A composition comprising an isolated DNA sequence encoding a mature P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 402.

4. A composition comprising an isolated DNA sequence encoding a soluble mature P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 310.

5. A composition comprising an isolated DNA sequence encoding a P-selectin ligand protein, said protein being characterized by the amino acid sequence set forth in SEQ ID NO:3.

6. An isolated DNA sequence capable of hybridizing under stringent conditions to the DNA sequence of any one of claims 1, 2, 3, 4 or 5.

7. The DNA sequence of any one of claims 1, 2, 3, 4, 5 or 6 operably linked to an expression control sequence.

8. A host cell transformed with the DNA of claim 7.

9. The host cell of claim 8, comprising a mammalian cell.

17. A composition comprising an antibody which specifically reacts with the P-selectin ligand protein of claim 13.

18. A composition comprising an antibody which specifically reacts with the protein of claim 15.

19. A method of identifying an inhibitor of P-selectin-mediated intercellular adhesion which comprises

(a) combining a P-selectin protein with a P-selectin ligand protein characterized by an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:1 from amino acid 1 to amino acid 402, the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 402, the amino acid sequence set forth in SEQ ID NO:1 from amino acid 42 to amino acid 310, and the amino acid sequence set forth in SEQ ID NO:3, said combination forming a first binding mixture;

(b) measuring the amount of binding between the P-selectin protein and the P-selectin ligand protein in the first binding mixture;

(c) combining a compound with the P-selectin protein and the P-selectin ligand protein to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

(e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture; wherein the compound is capable of inhibiting P-selectin-mediated intercellular adhesion when a decrease in the amount of binding of the second binding mixture occurs.

20. A method of treating an inflammatory disease which comprises administering a therapeutically effective amount of the composition of claim 14 to a mammal.